ULTRACYTOCHEMICAL STUDY ON GLUCOSE-6-PHOSPHATASE ACTIVITY IN HUMAN KIDNEY

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Received for publication January 22, 1975, and in revised form February 18, 1975

Glucose-6-phosphatase activity can be demonstrated ultracytochemically in surgical specimen of human kidney fixed by immersion in dilute aldehyde solution. Tissues fixed for 30 min. in 2% glutaraldehyde or 5 min. in 1% paraformaldehyde plus 3% glutaraldehyde solution and incubated for 15 min. in Wachstein-Meisel's medium at 37°C revealed satisfactory results.

The enzyme activity was confined to proximal convoluted tubule cells. Subcellular localizations were smooth and rough endoplasmic reticulum as well as nuclear envelopes. All the plasma membranes including brush borders showed no reaction products. Golgi apparatuses remained unreactive. The enzyme localizations in proximal tubule cells of human kidney were essentially same as those in hepatic parenchymal cells of various animals and in proximal tubule cells of rat kidney in the literature. This investigation showed that G6Pase can be used as a cytochemical marker for the human kidney study.

The ultracytochemical localization of glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase EC 3. 1. 3. 9.) (G6Pase) activity has been studied in various types of cells including parenchymal cells of liver (6, 12, 13, 21, 24) Kupffer cells as well as leucocytes in liver (18, 19), jejunal epithelial cells (7, 8, 9), pancreatic β cells (15), adreno-cortical cells (17), epithelial cells of endometrium (22), ganglionic cells of nervous system (23) as well as proximal convoluted tubule cells of the rat kidney (14). Few ultracytochemical study, however, has appeared in the past on the G6Pase activity of human surgical specimens except author's short communication on human kidney tissues (26, 27). This was probably due to the fact that the enzyme has been reported to be very sensitive to the fixation and easily lose its activity (4, 20, 24) and that requirements for ultracytochemical demonstration of the enzyme have also been reported to be rigid. Brief fixation by dilute aldehyde solution either by perfusion (4), or by transparenchymal perfusion (13) is necessary. Fresh frozen section followed by immersion in aldehyde solution for short periods of time using substrate protection was reported to be successful in rat liver and kidney by Kanamura (12, 14).

The perfusion fixation, however, cannot be applied to human tissues. The procedure using fresh frozen section proposed by Kanamura (12) is composed of many steps, which makes the method complicated and impractical.

This study was undertaken to know the possibility of ultracytochemical detection of G6Pase in human kidney tissues obtained by surgery, the optimal condition if detectable and finally the localizations of the enzyme in the tissues, which will serve as control findings for the pathological applications.

MATERIALS AND METHODS

Five human kidney tissues were used in this study. They were obtained in the form of biopsy materials in 3 cases and nephrectomy specimens in 2 cases. Renal tissues were cut into small pieces as soon as possible and fixed in ice-cold aldehyde solution by immersion for varying periods of time from 5 min. to 2 hours. Two types of fixatives were employed. One is 2% glutaraldehyde (GLA) in 0.1 M cacodylate buffer with 8% sucrose, and the other is 1% paraformaldehyde (PFA) and 3% GLA mixture in 0.07 M cacodylate buffer. After fixation tissues were rinsed overnight in 0.1 M cacodylate buffer containing 8% sucrose at 4°C. 40 μ frozen sections were cut on the freezing microtome and were incubated in Wachstein-Meisel's G6Pase medium (25) for 15–30 min. at 37°C. Tissue sections were osmicated, dehydrated in ethanol series and embedded in Epon 812 through propylene oxide.

As control, tissue sections were incubated in the medium from which glucose-6-phosphate (G6P) was omitted. In addition, G6P in the media was substituted by equal moles of glucose-1-phosphate, fructose-1, 6-diphosphate as well as 20 mM of β -glycerophosphate. Inhibition experiments were performed by adding 10 mM of sodium fluoride (NaF) and 1, 10, 50 mM of potassium cyanide (KCN) into the full incubating medium.

Ultrathin sections were cut with glass knives on LKB ultratome, stained with uranyl acetate and lead citrate and viewed by Hitachi HU 11B electron microscope.

RESULTS

Small block of kidney, at least less than 1 mm in one dimension preferably less than 0.5 mm, gave a good result. To obtain the wide area of observation and good orientation it is recommended to make thin slices of tissue before the immersion fixation.

Tissues fixed for 5 min. in 1% PFA and 3% GLA mixture solution or for 15–30 min. in 2% GLA solution, followed by 15 min. incubation yielded satisfactory enzyme localization.

G6Pase activity in human kidney tissues was demonstrated only in proximal convoluted tubule cells. Enzyme localizations were rough and smooth endoplasmic reticulum (ER) as well as nuclear envelopes (Fig. 1–4). Fine lead precipitates indicating G6Pase activity were found inside the smooth and rough ER and nuclear envelopes (Fig. 1 & 2). Tubular networks of smooth ER often located at the supranuclear region of the proximal tubule cells also contained reaction

Fig. 1. G6Pase activity is found in ER as well as in nuclear envelopes. Brush borders, and plasma membranes facing adjoining cell and peritubular basal lamina do not show the activity. A Golgi apparatus remains unreactive (arrow). Fixation; 5 min. in 1% PFA plus 3% GLA, incubation; 15 min. $\times 18,800$ A scale indicates one micron.

products (Fig. 3). No other organelles show the reaction. All the plasma membranes including brush borders were free from lead precipitates (Figs. 1-3). Golgi apparatuses composed of cisternae, vesicles and vacuoles were G6Pase negative (Fig. 4). Mitochondria, lysosome, and microbody remained unreactive.

When G6P was omitted from incubation medium, no reaction products were found in any portion of the cells (Fig. 5). Replacement of G6P either by equimolar fructose-1, 6-diphosphate (Fig. 6) or glucose-1-phosphate in the media brought the complete disappearance of the reaction products. When tissues were incubated in the media pH 6.7 in which 20 mM of β -glycerophosphate was dissolved in place of G6P, reaction products were not found in the ER system (Fig. 7) but found in Golgi apparatuses and lysosomes. The reaction in lysosomes and Golgi apparatuses by aforementioned medium with β -glycerophosphate was considered to be due to the non-specific acid phosphatase, since the reaction disappeared when pH of the medium was elevated from 6.7 to 7.0.

Cytochemical reaction was completely abolished by addition of 10 mM NaF in complete incubation media (Fig. 8). Marked diminution of the enzyme activities was observed in the tissue incubated with 10 mM and 50 mM of KCN.

DISCUSSION

Present study disclosed that G6Pase activity could be demonstrated consistently in human kidney tissues fixed briefly by immersion in aldehyde solution.

Enzyme activity was found in proximal convoluted tubule cells of the human kidney as was in accord with earlier histochemical works by Jacobsen et al. (10) and Jacobsen & Jørgensen (11) and cytochemical study of the rat kidney (14). Sub-cellular localizations were exclusively in ER system, namely smooth and rough ER and nuclear envelopes. The localizations were similar to those in hepatocytes of various animals (4, 6, 12, 13, 21, 24), jejunal epithelial cells (7, 8, 9), pancreatic β -cells (15), adreno-cortical cells (17) and ganglion cells (23). An active site of the enzyme at the cytomembrane of ER seems to be located at the inner face, since the reaction products accumulated in the ER cisternae and never went across the ER membrane into the cellular matrix. Biochemically, G6Pase has been localized in microsomal fraction, firmly attached to cytomembrane both in liver and kidney (1, 3, 16). It has been collected in particulate forms. Solubilization of the enzyme has been reported unsuccessful (2).

According to Kanamura (14), lead precipitates were consistently found not only in ER system but also at brush borders of the proximal tubule cells when the rat kidney tissue was incubated in Wachstein Meisel's G6Pase medium. Present study, however, revealed that the brush borders of the human proximal tubule cells have

FIG. 2. G6Pase activity is positive in ER as well as nuclear envelope. Note the absence of reaction products at brush borders and apical invaginations. Fixation and incubation; same as in Fig. 1. $\times 26,200$

FIG. 3. G6Pase activity in the tubular network of smooth ER is shown. Fixation and incubation; same as in Fig. 1. $\times 33,700$

FIG. 4. A Golgi apparatus is shown which is negative in G6Pase activity. Please note that a cisterna of smooth ER is positive among the negative Golgi lamellae. Rough ER is enzyme positive. Fixation and incubation; same as in Fig. 1. $\times 53,200$

never showed lead precipitates even when the tissues were incubated in G6Pase medium for long periods of time. Lead precipitates found at rat brush borders were interpreted as a result of non-specific hydrolysis of G6P by alkaline phosphatase (ALPase) (14). Similar membrane reaction was observed by Hugon et al. (7, 8, 9) at the striated borders as well as lateral cell walls of the jejunal epithelial cells when the tissues were incubated in G6Pase medium. The reaction has also been considered to be due to the non-specific ALPase at the plasma membrane.

This interpretation, however, brings up another problem because it means that the ALPase is active at pH 6.7 both at the brush borders of rat kidney and at the striated borders of the mouse small intestine. Yokoyama (28) observed that ALPase was consistently active at brush borders of human proximal convoluted tubule cells at alkaline pH. In the present study, human kidney tissues were incubated in the Wachstein and Meisel's medium at pH 6.7 as well as pH 7.0 in which 20 mM of β -glycerophosphate was dissolved instead of G6P to know whether the ALPase was active at these pH levels or not. The results clearly showed that ALPase of brush borders of human proximal convoluted tubules was not active both at pH 6.7 (figure, not shown) and at pH 7.0 (Fig. 7). The findings in the present study did not support their interpretations. To reconcile our findings and their interpretations, one should hypothesize the different pH dependency of ALPase activity among the species or among the tissues. Further study is warranted in this context.

Biochemical study of isolated brush borders from rabbit kidney disclosed no significant enrichment of G6Pase (5) and was interpreted that the brush borders were free from G6Pase. Present cytochemical study is in agreement with biochemical study of fractionated cell membrane (5). Both indicate that the plasma membrane is not the site of G6Pase localization.

It is worth while to note that immersion fixation in 2% GLA as well as in 1% PFA plus 3% GLA solution preserved G6Pase activity and good ultrastructure. The procedure performed in this study is simple and less complicated compared with fresh frozen section followed by immersion proposed by Kanamura (12). Many of these steps could be omitted and the morphology as well as enzyme localizations appeared satisfactory. This simplified method opened the possibility to use the G6Pase as a cytochemical marker for the human kidney study. Wide area of application in medicine includes not only renal disorders but also kidney involvements in systemic metabolic diseases. The ultracytochemical findings of G6Pase in normal human kidney tissue as revealed in this study constitute the control findings

FIG. 5. A tissue section incubated in the medium from which the substrate is deleted appears to have no reaction products. Fixation; 5 min. in 1% PFA plus 3% GLA, incubation; 15 min. $\times 26,600$

FIG. 6. The figure shows a tissue section incubated in the medium in which a substrate, 1.5mM G6P, is replaced by equal moles of fructose-1, 6-diphosphate. Absence of lead precipitates in ER system is apparent. Fixation and incubation; same as in Fig. 5. $\times 40,000$

FIG. 7. When the tissues are incubated in the medium at pH 7.0, with a 20mM of β -glycerophosphate as a substrate, no reaction products are found both at brush borders and in ER. Fixation and incubation; same as Fig. 5. $\times 26,600$

FIG. 8. Chemical inhibition of G6Pase activity is achieved by addition of 10mM of NaF to the full incubating medium. Complete disappearance of reaction is apparent. Fixation; 30 min. in 2% GLA, incubation; 15 min. $\times 26,600$

in themselves when a pathological application is intended.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. S. Fukuda and Mr. E. Sato for their technical assistances and to Mrs. M. Yokoyama for preparation of the manuscript.

This work was partly supported by grant in aid from Ministry of Education 867106 in 1973.

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